New Nucleotide Sequences Coding for the thrE Gene and Process for the Enzymatic Production of L-threonine using Coryneform Bacteria

Patent Claims

1. Preferably recombinant DNA derived from Corynebacterium and replicable in coryneform microorganisms, which contains at least one nucleotide sequence that codes for the thrE gene.

2. Replicable DNA according to claim 1 with

   (i) the nucleotide sequences shown in SEQ-ID-No. 1, or SEQ-ID No. 3, which code for the thrE gene, or

   (ii) at least once sequence that corresponds to the sequences (i) within the degeneration region of the genetic code, or

   (iii) at least once sequence that hybridises with the sequences complementary to the sequences (i) or (ii), and/or optionally

   (iv) functionally neutral sense mutations in (i).

3. Amino acid sequence of the protein, derived from the nucleotide sequences according to claim 1 or 2, shown in SEQ-ID-No. 2 and in SEQ-ID-No. 4.

4. Coryneforme microorganisms, in particular of the genus Corynebacterium, transformed by the introduction of one or more of the replicable DNA according to claim 1 or 2.

5. Corynebacterium glutamicum DM368-2 pZ1thrE, filed under Number DSM 12840.

6. Process for producing L-threonine by fermentation of coryneform bacteria, characterised in that bacteria are used in which nucleotide sequences coding for the thrE

gene are amplified, and in particular are overexpressed.

7. Process according to claim 6, characterised in that bacteria are used in which in addition one or more genes of the threonine biosynthesis pathway is/are amplified.

8. Process according to claims 6 and 7, characterised in that a strain transformed with a plasmid vector is used and the plasmid vector carries the nucleotide sequence coding for the thrE gene.

9. Process according to claims 6 and 8, characterised in that the thrE gene is overexpressed in microorganisms that contain further metabolite or antimetabolite resistance mutations.

10. Process according to claims 6 to 9, characterised in that the microorganisms in order to achieve over-expression are fermented in altered culture media, and/or the fermentation conditions are changed.

11. Process according to claims 6 to 10, characterised in that microorganisms are used in which the metabolic pathways that reduce threonine formation are at least partially switched off.

12. Process according to claims 6 to 11, characterised in that microorganisms are used in which in addition to the thrE gene the remaining genes of the metabolic pathway for threonine formation are amplified individually or jointly (overexpressed).

13. Process for producing L-threonine, characterised in that the following steps are carried out:

a) Fermentation of microorganisms according to one or more of the preceding claims, in which at least the thrE gene is amplified (overexpressed) optionally in combination with further genes,

b) Enrichment of the L-threonine in the medium or in the cells of the microorganisms, and

c) Isolation of the L-threonine.

14. Process according to one or more of the preceding claims, characterised in that microorganisms of the genus Corynebacterium are used.

15. Process for isolating the thrE gene, characterised in that mutants, preferably of coryneform bacteria, defective in the thrE gene that do not grow or grow only weakly on a nutrient medium containing a threonine-containing oligopeptide are obtained as indicator strains, and

a) the thrE gene is identified and isolated after establishing a gene bank, or

b) in the case of transposon mutagenesis is selected for the transposon preferably exhibiting resistance to antibiotics, and the thrE gene is thereby obtained.

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